ISSN 1346-7565
Acta Phytotax. Geobot. 55 (3): 167–180 (2004)

Close Relationship between Mexican and Chinese *Magnolia* (Subtropical Disjunct of Magnoliaceae) Inferred from Molecular and Floral Scent Analyses

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The floral scents of *Magnolia coco* (distributed in Asia) and *M. mexicana* (New World), plus two Mexican *Magnolia* taxa, *M. schiedeana* and *M. dealbata*, were analyzed using GC-MS. A molecular phylogenetic analysis was also conducted using a combined data set of plastid DNA sequences obtained from GenBank to construct a well-resolved phylogenetic tree. The analysis indicated a sister relationship between subtropical-tropical Asian and American disjunct groups (including *M. coco* and *M. mexicana*) which was not shown in previous studies. Entire flowers and floral parts (sepals, petals, and gynoecia) of *M. coco* emitted methyl 2-methylbutanoate (83-96%), whereas entire flowers of *M. mexicana* emitted α-farnesene (67%) which was mainly emitted from the sepals. The petals and gynoecium of *M. mexicana* emitted methyl 2-methylbutanoate as the main component with relatively small amount of α-farnesene. The common presence of methyl 2-methylbutanoate in the petals and gynoecium of both taxa may represent an ancestral scent profile because subtropical-tropical disjunct groups, which include *M. coco* in one and *M. mexicana* in the other, phylogenetically show a sister relationship. The floral scent of *M. schiedeana* was exclusively composed of monoterpenes, especially geranyl methyl ether (87%). The floral scent of *M. dealbata* was predominantly composed of benzenoids, especially 2-phenylethanol (78%) and methyl phenylacetate (13%).

Key words: *atpB-rbcL*, biogeography, disjunction, fragrance, *Gwillimia*, *ndhF*, *psbA-trnH*, *Talauma*, *trnK*, *matK*

Extant taxa of Magnoliaceae (approximately 220 species of trees and shrubs) exhibit a classic pattern of temperate (eastern Asia - eastern North America) and subtropical-tropical (Southeast Asia - Central and northern South America) disjunct distributions (Li 1952, Takhtajan 1969, Wu 1983, Nooteboom 1993, Frodin & Govaerts 1996, Figlar & Nooteboom 2004). A recent molecular phylogenetic analysis, and paleobotanical and geological evidences indicated that the subtropical-tropical disjuncts of Magnoliaceae occurred in the mid to late Eocene (~36 million years ago), earlier than the temperate disjunction (28-25 mya; Azuma *et al.* 2001a).

Floral scent is an important pollinator attractant in most species of Magnoliaceae and other flowering plants (van der Pijl 1960, 1961, Pellmyr &

Thien 1986, Proctor *et al.* 1996, Gottsberger 1999, Thien *et al.* 2000). The flowers of Magnoliaceae emit a large number of volatile compounds including terpenoids, benzenoids, fatty acid derivatives, and nitrogen-containing compounds (Thien *et al.* 1975, Yasukawa *et al.* 1992, Azuma *et al.* 1997, 2001b). The floral scent of each taxon, however, is usually dominated only by one or two compounds (or class of compounds).

Morphologically and molecular phylogenetically closely related *Magnolia* taxa disjunctly distributed in temperate eastern Asia and eastern North America share similar scent profiles, e.g., *M. obovata* Thunb. (= *M. hypoleuca* Siebold et Zucc.) in Japan and *M. tripetala* (L.) L. in North America both strongly emit methyl benzoate as the main compound (Azuma *et al.* 1997). This pattern is also exhibited by *M. denudata* Desr. [= *M. heptapeta* (Buc'hoz) Dandy] and *M. liliiflora* Desr. [= *M. quinquepeta* (Buc'hoz) Dandy] in China, and *M. acuminata* (L.) L. in North America, all three emitting pentadecane as the main scent compound (Thien *et al.* 1975, Boland & Gäbler 1989, Azuma *et al.* 1997).

We have been interested in whether or not floral scents of subtropical-tropical disjunct species of Magnoliaceae also exhibit similar scent profiles. In this study, we conducted chemical analyses of floral scents from representative taxa of the subtropical-tropical disjuncts, e.g., *Magnolia coco* (Lour.) DC. (Asian sect. *Gwillimia*) and *M. mexicana* DC. (American sect. *Talauma*) [= *Talauma mexicana* (DC.) G. Don]. In addition, we analyzed floral scents of two Mexican species previously not analyzed; *M. schiedeana* Schltl. (sect. *Magnolia*) and *M. dealbata* Zucc. (sect. *Macrophylla*).

A new classification system of Magnolioideae (Magnoliaceae) is recently given, based on the latest available data on DNA and morphology (Figlar & Nooteboom 2004). Therefore, we followed the new system throughout this study.

Recent plastid DNA-based phylogenetic analyses which were independently conducted by Azuma *et al.* (2001a) and Kim *et al.* (2001) did not show a sister relationship between subtropical-tropical Asian and American disjunct groups. Thus we analyzed the combined sequence data provided by the previous two studies, e.g., the *trnK* intron including *matK* region and two intergenic spacer regions (*atpB-rbcL* and *psbA-trnH*) by Azuma *et al.* (2001a) and the *ndhF* region by Kim *et al.* (2001), to construct a well-resolved phylogenetic tree. The combined data matrix contains 36 representative taxa of the family (34 ingroups and two outgroups) common to both previous studies.

Materials and Methods

Molecular phylogenetic analysis

All of the plastid DNA sequence data used in this study was obtained from the GenBank (see Appendix for the accession numbers). The combined sequence data were manually aligned. Indels (insertions and/or deletions) were scored as additional binary characters (0 or 1) except the length mutations in polynucleotide tracks which were ignored. Three short sequence inversions were recorded in the *matK* coding region and one in the *psbA-trnH* spacer region. These inversions were excluded from the data matrix (see Azuma *et al.* 1999). The final data matrix was composed of 5971 characters including twelve indels. Two *Liriodendron* species (*L. tulipifera* and *L. chinense*) were used as outgroups (Azuma *et al.* 2001a).

A maximum parsimony analysis was conducted using PAUP* 4.0b10 (Swofford 2002). A heuristic search with one hundred random-addition sequence replicates with TBR (Tree Bisection Reconnection) branch-swapping was carried out. Bootstrap analysis with 1000 replicates (Felsenstein 1985) and Bremer support analysis (Bremer 1988) were conducted to measure confidence levels of each branch in the phylogeny. We used AutoDecay, version 4.0 (Eriksson 1998) to compute Bremer support.

Taxa studied for floral scent chemistry

Magnolia coco, the type species of sect. *Gwillimia*, occurs in S. China, Taiwan, and N. Vietnam (Frodin & Govaerts 1996). The small evergreen trees or shrubs are 2-4 m high, with small nodding flowers (3-5 cm in diam.) that open in the evening and emit a sweet scent. Flowers close by midnight and do not reopen and by the second day afternoon or evening all sepals, tepals and stamens are detached (Figlar 1985, Azuma pers. observ.). The sepals and petals exhibit little variation in size and shape. The thin sepals are usually greenish, and the fleshy petals are white-cream colored (Chen & Nooteboom 1993). Stone (1966) and Treseder (1978) pointed out that the flower of *M. coco* has nectaries or nectar glands. However, one of the authors (H. A.) could not find any nectar production in the flowers.

Magnolia section *Talauma* is distributed throughout subtropical and tropical regions of the Americas including the West Indies (Frodin & Govaerts 1996, Figlar & Nooteboom 2004). *Magnolia mexicana* is found in Mexico, Guatemala, and Honduras (Frodin & Govaerts 1996). The evergreen trees are about 3-20 m (or more) in height, and produce relatively large flowers (about 10-15 cm in diam.) on terminal branches (Hernández-Cerda 1980). The flower may be functional for a couple of days like most species of Magnoliaceae. The thin sepals are brown, and the hard fleshy petals are cream-colored. The flowers are borne upright on the plant and emit a heavy scent.

Magnolia schiedeana (sect. *Magnolia*) is also endemic to Mexico. The molecular phylogenetic analysis indicated that *M. schiedeana* was closely related to *M. grandiflora* L. (SE United States to Mexico) and *M. tamaulipana* Vázquez (endemic to Mexico) (Azuma *et al.* 2001a). These evergreen plants produce medium-large flowers (5-20 cm wide) with moderate amounts of scent.

Magnolia dealbata (sect. *Macrophylla*), endemic to Mexico and the West Indies (Frodin & Govaerts 1996), is closely related to *M. macrophylla* Michx. in eastern North America (Azuma *et al.* 2001a). The deciduous, tall tree produces large (10-20 cm in diam.), heavily scented, vase-shaped flowers (Hernández-Cerda 1980).

Sampling and chemical analysis of floral scent

Floral scent of *Magnolia coco* was collected from *in situ* plants cultivated in Kyoto, Japan, from July 10 to 16, 2001. Sampling took place between 17:00-18:00 when the flowers began to open and emit a sweet scent. The floral scents of *M. mexicana*, *M. schiedeana*, and *M. dealbata* were collected from plants growing in natural fields, coffee plantations and gardens in Coatepec, Veracruz, Mexico, or in the city itself near the Instituto de Ecología, Xalapa, from April 21 to May 4, 1998. Detached female-stage flowers were placed in containers with water and the scent collection started between 12:00-14:00. To evaluate the contribution of each floral part versus the scent produced by the whole flower, volatiles emitted from detached sepals (three outer tepals/sample), petals (six inner tepals/sample), and gynoecium with appressed anthers (one /sample) of *M. coco* and *M. mexicana* were collected separately. Vouchers are housed in the herbarium at Tulane University (see Azuma *et al.* 2001a) and KYO.

An intact flower or floral part (from one flower) was enclosed in a polyethylene bag (25 cm × 35 cm). A mini-pump (Model MP-2N, SIBATA Scientific Instrument, Tokyo) was used to extract air from around enclosed flowers. A glass cartridge (7 mm × 5 cm) containing adsorbent [50 mg Tenax TA (30 mg Tenax GR for *Magnolia coco*), Mesh 60/80, GL Sciences, Tokyo] was inserted into the flow line (Silicon tubing). The pump was driven at approximately 500 ml (150 ml for *M. coco*) min^{-1} for 2 hours. The cartridges with trapped volatiles were sealed in aluminum foil and stored in a -20°C freezer. Blanks (controls) were simultaneously collected from empty bags.

Volatile compounds were eluted from the adsorbent with 500 μl (200 μl for *Magnolia coco*) of diethyl ether. Ten μl of nonyl acetate solution (0.5 mg ml^{-1} dichloromethane) was added to all samples as an internal standard to calculate the amounts of volatile compounds. Concentration of the eluent was not conducted. Two μl of eluents were used for gas chromatography-mass spectrometry (GC-MS) analysis performed with a Hewlett-Packard 5890 SERIES II gas chromatograph (Agilent Technologies, CA, U.S.A.) coupled with a Hewlett-Packard 5971A mass selective detector. A fused silica capillary column (DB-17, 30 m, 0.25 mm inner diameter, 0.25 μm film thickness) of intermediate polarity was used. The injection temperature was 250°C. The temperature was kept at 50°C for the first 5 min, programmed 5°C min^{-1} to 150°C, and then raised 10°C min^{-1} to 250°C and held for 10 min. Helium was used as the carrier gas. Identifications of volatiles were made by comparing their mass spectra with those in the computer library (including our original library) and/or with GC-retention times and MS-spectra of the authentic compounds.

Results

Molecular phylogenetic analysis

There were 396 (6.6%) variable characters in the data matrix, of which 212 (3.6%) characters were parsimony informative. Within the ingroup (subfamily Magnolioideae), there were 282 (4.7%) variable characters, of which 125 (2.1%) characters were informative. The maximum parsimony analysis generated 30 equally most parsimonious trees (461 steps, CI excluding uninformative characters = 0.83, RI = 0.89, and RC = 0.80). One of 30 most parsimonious trees is shown in Fig. 1 with bootstrap percentages and Bremer support.

Floral scent chemistry of Magnolia coco *and* M. mexicana

The chemical compositions of floral scents emitted by *Magnolia coco* and *M. mexicana* are shown in Table 1. Volatile compounds detected are classified into four groups according to similarities in chemical structure, i.e., fatty acid derivatives (branched and linear types), benzenoids, terpenoids (monoterpenes and sesquiterpenes), and nitrogen-containing compounds (Table 1).

Floral scent emitted from a whole flower of *Magnolia coco* was dominated by methyl 2-methylbutanoate (93.3%) with a small amount of a related compound, methyl 2-methyl-2-butenoate (6.7%) (Table 1). Volatiles emitted from separate floral parts (sepals, petals, and gynoecium) displayed almost identical chemical profiles with those of the whole flower. The petals were the main source for the emission of methyl 2-methylbutanoate in *M. coco* (Table 1).

Floral scent emitted from a whole flower of *Magnolia mexicana* was largely dominated by α-farnesene (66.9%), followed by *trans*-β-ocimene (9.5%), linalool (9.4%), methyl 2-methylbutanoate (4.5%), and indole (4.5%) (Table 1). In contrast to *M. coco*, volatiles emitted from separate floral parts (sepals, petals, and gynoecium) showed different chemical profiles from that of the whole flower (Table 1). The sepals emitted α-farnesene (69.1%) and a few minor compounds that the whole flower also emitted, but they lacked methyl 2-methylbutanoate. On the other hand, in the scent from petals methyl 2-methylbutanoate (62.5%) was dominant, and α-farnesene (10.6%) was a minor component (Table 1). Although the gynoecium was almost scentless, methyl 2-methylbutanoate (76.4%) was also a main component and α-farnesene was detected in a trace amount.

Floral scent chemistry of Magnolia schiedeana *and* M. dealbata

Floral scent of *Magnolia schiedeana* was predominantly composed of terpenoids (monoterpenes, 100%), especially geranyl methyl ether (86.8%),

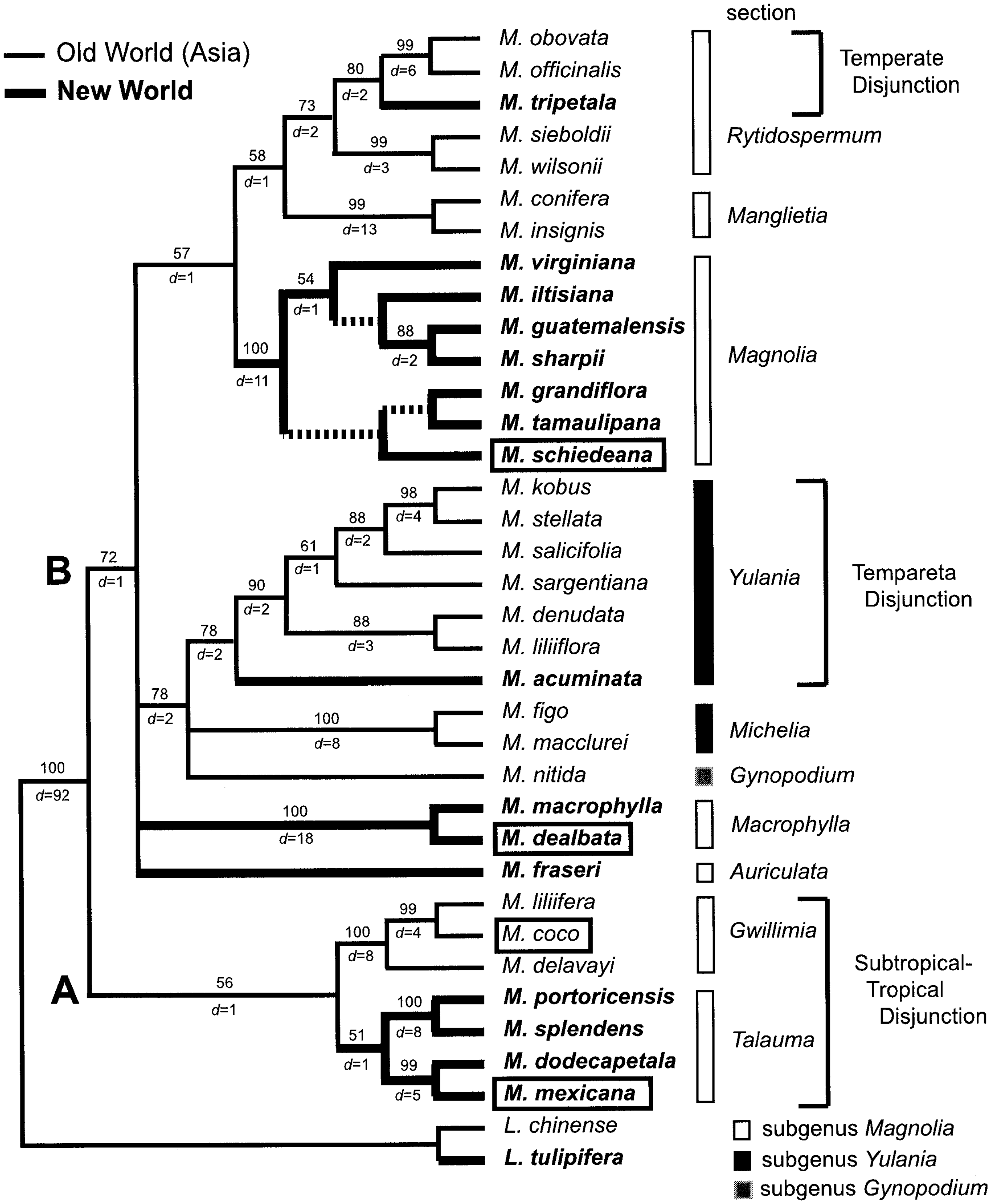

FIG. 1. One of 30 most parsimonious trees (461 steps, CI excluding uninformative characters = 0.83, RI = 0.89, and RC = 0.80) of Magnoliaceae based on combined plastid DNA sequence data (*trnK* intron including *matK* region, *atpB-rbcL* and *psbA-trnH* intergenic region, and *ndhF* region). The numbers above the branches indicate bootstrap percentages. The numbers below the branches are the Bremer support (*d*). The strict consensus tree is identical to the tree collapsed at the dashed branches. Geographical distributions (Old World or New World), sectional treatments within *Magnolia* (sensu Figlar & Nooteboom 2004), and temperate and subtropical-tropical disjuncts are also indicated. Boxed species indicate those included in scent analysis of this study.

TABLE 1. Chemical composition and mean percentage [min.-max.] of volatile compounds detected in floral scent from a whole flower or floral part of *Magnolia coco* and *M. mexicana*. Compounds marked with an asterisk (*) were identified by comparison with mass spectra and GC-retention times of authentic compounds. - = not detected; + = trace amount.

Taxa	*M. coco*			
A whole flower or floral part	**Whole**	Sepals	Petals	Gynoe.
Number of sample (individual)	**4 (4)**	3 (3)	3 (3)	3 (3)
Total amount (average µg/hour) (µg min.-max.)	**43.5** **(30.6-56.8)**	7 (1.7-10.6)	37.8 (15.5-49.5)	9.2 (5.5-14.9)
FATTY ACID DERIVATIVES				
(Branched types)				
Methyl 2-methylbutanoate*	**93.3** **[91.6-95.3]**	95.7 [92.8-99.6]	92.1 [83.3-97.9]	82.9 [57.1-96.3]
Methyl 2-methyl-2-butenoate*	**6.7** **[4.6-8.2]**	1.8 [0.0-5.0]	6.8 [2.0-14.0]	2.6 [1.3-3.3]
(Linear types)				
Methyl butanoate*	+ **[0.0-0.1]**	-	-	+ [0.0-0.1]
Methyl hexanoate*	**0.1** **[0.1-0.1]**	-	0.1 [0.1-0.1]	-
BENZENOIDS				
Benzyl alcohol*	-	-	-	-
Methyl salicylate*	+ **[0.0-0.1]**	-	+ [0.0-0.1]	-
TERPENOIDS				
(Monoterpenes)				
cis-β-Ocimene*	-	-	-	-
trans-β-Ocimene*	-	+ [0.0-0.1]	0.2 [0.0-0.7]	1.4 [0.0-4.1]
Linalool*	-	-	-	0.3 [0.0-0.5]
(Sesquiterpenes)				
4,8-Dimethyl-1,3(*E*),7-nonatriene*	+ **[0.0-0.1]**	1.0 [0.0-2.7]	0.5 [0.0-1.4]	1.7 [0.0-4.8]
α-Farnesene*	-	-	-	-
β-Farnesene*	-	-	-	-
β-Elemene*	-	-	-	-
Caryophyllene*	-	-	-	0.3 [0.0-0.9]
Germacrene D*	-	1.6 [0.0-4.5]	0.3 [0.0-0.7]	8.3 [1.0-22.7]
Germacrene B*	-	-	-	0.4 [0.0-1.2]
trans-Nerolidol*	-	-	+ [0.0-0.1]	1.9 [0.0-5.4]
NITROGEN-CONTAINING COMPOUND				
Indole*	-	-	-	-
UNKNOWNS				
unknown	-	-	-	-
unknown	-	-	-	-
unknown	-	-	-	-
unknown	+ **[0.0-0.1]**	-	+ [0.0-0.1]	-
TOTAL (%)	**100**	100	100	100

M. mexicana			
Whole	Sepals	Petals	Gynoe.
3 (3)	1 (1)	1 (1)	1 (1)
233.6 **(123.1-354.1)**	221.5 -	97.4 -	3.4 -
4.5 **[3.5-8.1]**	-	62.5	76.4
-	-	-	-
-	-	-	-
-	-	-	-
1.9 **[0.6-2.4]**	3.9	-	-
0.3 **[0.0-0.4]**	0.4	-	-
0.5 **[0.4-0.6]**	0.4	+	+
9.5 **[8.0-10.4]**	8.1	0.4	14.7
9.4 **[8.7-12.2]**	10.8	15.5	-
1.0 **[0.8-1.7]**	1.3	-	-
66.9 **[62.0-70.1]**	69.1	10.6	+
1.3 **[1.0-1.9]**	1.1	-	-
-	-	-	8.8
+ **[0.0-0.1]**	+	-	-
-	-	-	-
-	-	-	-
-	-	-	-
4.5 **[3.6-5.3]**	4.8	11.1	-
+ **[0.0-0.1]**	+	-	-
+ **[0.0-0.1]**	+	-	-
0.3 **[0-0.6]**	-	-	-
-	-	-	-
100	100	100	100

β-myrcene (8.0%), and limonene (3.4%) (Table 2). Most of monoterpenes detected are commonly present in floral scents of many flowering plants, but the geranyl methyl ether (the main component) rarely occurs in natural floral scents (Knudsen *et al.* 1993).

Flower of *Magnolia dealbata* emitted large amount of volatiles, and the scent was dominated by benzenoids (ca. 95%), i.e., 2-phenylethanol (77.9%), methyl phenylacetate (12.9%), and 2-phenylethyl acetate (3.8%) (Table 2). These benzenoids are common in floral scents of many flowering plants (Knudsen *et al.* 1993).

Discussion

Molecular phylogenetic analysis

There are two large clades in the molecular phylogenetic tree (clade A and B in Fig. 1). Clade A is composed of subtropical-tropical Asian and American taxa representative of sections *Gwillimia* (ca. 16 spp. in Asia) and *Talauma* (ca. 55 spp. in Americas). The sister relationship between the subtropical-tropical Asian and American disjunct groups is newly suggested by this analysis.

Morphologically, subtropical-tropical southeastern Asian section *Gwillimia* and American section *Talauma* are very similar (Nooteboom 1985, 1987). This newly detected sister relationship of subtropical-tropical disjunct groups fits the traditional and recent classification schemes based on morphological characters (Nooteboom 1985, 1987, Figlar & Nooteboom 2004). Although the sister relationship was weakly supported by the bootstrap analysis (56%), available molecular and morphological data indicate the subtropical-tropical Asian and American disjunct groups to be monophyletic.

Clade B is composed mainly of temperate taxa, and the topology is almost identical to that previously reported by Azuma *et al.* (2001a). In the previous study, the sister relationship between *Magnolia tripetala* (North America) and its Asian

TABLE 2. Chemical composition and mean percentage of volatile compounds detected in floral scent from a whole flower of Mexican *Magnolia*. Compounds marked with an asterisk (*) were identified by comparison with mass spectra and GC-retention times of authentic compounds. - = not detected; + = trace amount.

Taxa	*M. schiedeana*		*M. dealbata*	
A whole flower or floral part	Whole		Whole	
Number of sample (individual)	2 (1)		2 (1)	
Total amount (average μg/hour)	173.6		909.3	
[flower-1, 2, μg/sample]	[238.7, 108.4]		[798.1, 1020.5]	
BENZENOIDS				
Methyl benzoate*	-		+	[+, 0.1]
2-Phenylethanol*	-		77.9	[77.1, 78.7]
Methyl phenylacetate*	-		12.9	[14.3, 11.5]
2-Phenylethyl acetate*	-		3.8	[3.9, 3.6]
TERPENOIDS				
(Monoterpenes)				
β-Pinene*	0.2	[0.1, 0.2]	-	
β-Myrcene*	8.0	[3.3, 12.6]	-	
Limonene*	3.4	[1.4, 5.4]	-	
cis-β-Ocimene*	+	[0.1, +]	-	
trans-β-Ocimene*	1.0	[0.9, 1.1]	-	
Terpinolene*	0.5	[0.3, 0.6]	-	
Geranyl methyl ether*	86.8	[93.5, 80.1]	-	
Methyl geranylate*	0.1	[0.2, +]	-	
1,8-Cineole*	-		+	[+, +]
Linalool*	-		2.0	[2.0, 2.0]
(Sesquiterpenes)				
β-Elemene*	-		0.7	[0.8, 0.6]
Caryophyllene*	-		0.5	[0.5, 0.4]
Humulene*	-		0.4	[0.3, 0.4]
Germacrene D	-		0.8	[0.5, 1.1]
Germacrene B	-		+	[+, +]
sesquiterpene	-		0.1	[0.1, 0.1]
sesquiterpene	-		+	[+, +]
sesquiterpene	-		+	[+, +]
sesquiterpene	-		0.9	[0.4, 1.3]
UNKNOWNS				
unknown	-		0.1	[0.1, 0.1]
unknown	-		+	[+, +]
unknown	+	[0.1, +]	-	
TOTAL (%)	100		100	

counterparts (*M. obovata* and *M. officinalis*, a temperate disjunct) was not evident (Azuma *et al.* 2001a) or weakly supported (Kim *et al.* 2001). The tree obtained here clearly shows the sister relationship between them with a moderate bootstrap value (80%; Fig. 1). Similarly, a sister relationship in another temperate disjunct, i.e., *M. acuminata* vs. Asian counterparts, was weakly supported in the previous studies (52% and 67% bootstrap value), but the present analysis has a relatively high bootstrap percentage of 78% (Fig. 1).

Because the tree topology generated in this study is well concordant with previous molecular, morphological and taxonomic studies of Magnoliaceae and the confidence levels of several relationships (clades) by bootstrap analysis are improved, we consider the tree obtained here to be a well-resolved and reliable phylogeny of Magnoliaceae by this time.

Floral scent chemistry of Magnolia coco *and* M. mexicana

Comparison of the floral scent profiles of *Magnolia coco* (Asian sect. *Gwillimia*) with *M. mexicana* (American sect. *Talauma*) indicate methyl 2-methylbutanoate is the only major compound shared by the two species (Table 1). The sepals of *M. mexicana* are the main source for scent emission in quantity and seem to be specialized to emit α-farnesene (Table 1).

More than 80 chemicals have been identified in the floral scents of 24 taxa (except taxa studied here) of Magnoliaceae (Thien *et al.* 1975, Yasukawa *et al.* 1992, Azuma *et al.* 1997, 2001b). However, methyl 2-methylbutanoate occurs only in two taxa as a minor component, i.e., *Magnolia hypoleuca* (= *M. obovata*) (2.5%) and *Michelia compressa* (0.2%).

As noted, morphological and molecular phylogenetic studies indicate that the southeast Asian sect. *Gwillimia* and the American sect. *Talauma* are closely related groups (clade A in Fig. 1). We hypothesize that the presence of methyl 2-methylbutanoate in floral scents of *Magnolia coco* and *M. mexicana* may represent a portion of an ancestral scent profile emitted from the common ancestor of the two lineages (as found in some temperate disjunct taxa of Magnoliaceae). To confirm this hypothesis, additional data for floral scent chemistry of other members of tropical disjunct groups are needed.

Although methyl 2-methylbutanoate is a unique chemical in the floral scent of the Magnoliaceae, it occurs in other families of basal angiosperms as a dominant component. For example, in the floral scent of *Victoria amazonica* x *cruziana* (Nymphaeaceae; Kite *et al.* 1991), and in *Eupomatia laurina* (Eupomatiaceae; Bergström *et al.* 1991). In addition, other short-branched fatty acid esters occur as major components in the scents of *E. bennettii* (Eupomatiaceae; Bergström *et al.* 1991) and some taxa of *Anaxagorea* (Annonaceae; Jürgens *et al.* 2000), and in the volatiles from scented female cones of *Cycas rumphii* (Cycadaceae; Pellmyr *et al.* 1991).

It is interesting that Nymphaeaceae, Eupomatiaceae, Annonaceae, and Magnoliaceae are essentially woody subtropical-tropical ancient lineages of angiosperms (Qiu *et al.* 1999, Soltis *et al.* 2000) and attract beetles for pollination (Bernhardt & Thien 1987, Bernhardt 2000, Thien *et al.* 2000). These suggest that methyl 2-methylbutanoate and/or short-branched fatty acid esters may have played an important role in ancient tropical beetle pollination systems, although the pollination biology of southeastern Asian *Gwillimia* and the American *Talauma* taxa has not been well studied.

The pollination biology of *Magnolia coco* and *M. mexicana* is unknown but floral biology of the two species should be quite different. *Magnolia coco* produces night-blooming nodding flowers which may be functional for one night, whereas *M. mexicana* flowers for a couple of days. Flower-visitors for *M. coco* and *M. mexicana* are unknown. Apparently, floral scent emission is important in the nocturnal flowers of *M. coco*. *Magnolia ovata* (A. St.-Hil.) Spreng. (= *Talauma ovata* A. St.-Hil.), a close relative to *M. mexicana* in Brazil (Azuma *et al.* 2001a), is known to be exclusively pollinated by a beetle, *Augoderia nitidula* (Dynastidae) (Gibbs *et al.* 1977).

If methyl 2-methylbutanoate was the main scent component in an ancestral lineage of *Magnolia mexicana*, relatively strong emission of α-farnesene from the sepals may results from interaction with certain pollinator or environment condition after disjunction of the ancestral lineage to both continents. Field observation of pollinator and floral biology of *M. mexicana* with chemical data of the scent are critically needed to confirm this hypothesis.

Floral scent chemistry of Magnolia schiedeana

Molecular phylogenetic analysis indicates that *Magnolia schiedeana* (sect. *Magnolia*) is closely related to *M. grandiflora* and *M. tamaulipana* rather than to other members of sect. *Magnolia* as suggested by morphological features (Fig. 1; Treseder 1978, Vázquez-G. 1994, Azuma *et al.* 1999, 2001a). *Magnolia schiedeana* and *M. grandiflora* are known to be hexaploid (2n=114) (Chen *et al.* 2000), implying reticulate evolution occurred in sect. *Magnolia*, perhaps accounting for the low resolution of relationships in the section (Fig. 1).

The floral scent chemistry of the three species (*Magnolia schiedeana*, *M. grandiflora*, and *M. tamaulipana*) also supports a close taxonomic affinity. The chemical profile of *M. schiedeana* (endemic to middle-eastern Mexico; Vázquez-G. 1994) is very similar to that of *M. tamaulipana* (endemic to deciduous cloud forest in Tamaulipas, Mexico; Vázquez-G. 1994). The dominant scent compound in both species is geranyl methyl ether (87% and 86%), which does not occur in any other member of the Magnoliaceae that have been analyzed for floral scents (Table 1; Azuma *et al.* 1997). On the other hand, *M. grandiflora*, widely distributed in the southern North America, emits a mixture of volatiles, geraniol (20%), *trans*-β-ocimene (14%), β-myrcene (13%), and methyl dodecanoate (13%) (Azuma *et al.* 1997). These volatiles are very common in other flowering plants (Knudsen *et al.* 1993).

The chemical composition of *Magnolia grandiflora* is different from those of *M. schiedeana* and *M. tamaulipana*. Based on the chemical structures, however, geranyl methyl ether is probably derived from geraniol by addition of a methyl group. Therefore, we consider the floral scents of the three species essentially consist of the same class of chemicals, i.e., geraniol and its derivative.

The flowers of *Magnolia schiedeana* and *M. tamaulipana* are pollinated by only a few species of beetles. *Magnolia schiedeana* are pollinated by *Stenagria* spp. (Staphylinidae: Aleocharinae) and *Cyclocephala jalapensis* (Scarabaeidae: Dynastinae) (Dieringer & Espinosa S. 1994). Likewise, *M. tamaulipana* are exclusively visited by *Cyclocephala caelestis* (Scarabaeidae: Dynastinae) and *Myrmecocephalus* sp. (Staphylinidae) (Dieringer *et al.* 1999). Whereas flowers of *M. grandiflora* attract a wide variety of insects including beetles, flies, and bees (Thien 1974, Peigler 1988). These field observations suggest that specialized emission of a single compound in *M. schiedeana* and *M. tamaulipana* seems to be related to the specialized pollinators.

Floral scent chemistry of Magnolia dealbata

The chemical profile of *Magnolia dealbata* greatly differs from a closely related taxa, *M. macrophylla* in central to eastern North America. The floral scent of *M. macrophylla* ssp. *macrophylla* and ssp. *ashei* mainly consisted of sesquiterpenes and fatty acid methyl esters (Thien *et al.* 1975).

Similar qualitative difference between closely related taxa is observed between *Magnolia virginiana*-1 and *M. virginiana*-2 (Azuma *et al.* 1997). The floral scent of *M. virginiana*-1 collected from plants in the southern USA was composed mainly of terpenoids (linalool, 47%) and fatty acid methyl esters (methyl decanoate, 16%; methyl dodecanoate, 14%; methyl decenoate, 8%) as in *M. macrophylla* (Thien *et al.* 1975). The scent of *M. virginiana*-2 collected in the northeastern USA was composed of benzenoids; 2-phenylethanol (53%), methyl phenylacetate (14%), which are the same compounds in the scent of *M. dealbata* (Table 1).

It is known that floral scent chemistry is often variable at intraspecific level (Gregg 1983, Groth *et al.* 1987; Moya & Ackerman 1993, Tollsten &

Bergström 1993, Azuma *et al.* 2001b), and environment conditions (light, temperature, and humidity) may also affect the chemical composition or emission rate of volatiles to some extent (Nielsen *et al.* 1995). Therefore, more detailed comparative studies of floral scent chemistry with bioassay using the authentic compounds and observation of flower-visitors at inter- and intraspecific levels are needed in taxa studied and other members of subtropical-tropical groups for better understanding the evolutionary trends of floral scent chemistry and flower-pollinator interaction in Magnoliaceae.

Conclusions

It is thought that chemical profiles of floral scent reflect interactions with pollen-vectors (pollinators) and/or floral phenology (e.g., nocturnal flowering). Indeed, some scent compounds may result from interaction between flowers and pollinators, e.g., sulfur-containing compounds in bat-pollinated plants (Knudsen & Tollsten 1995, Bestmann *et al.* 1997). However, not all of scent compounds may be derived from flower-pollinator interaction. Although it may be difficult to distinguish these volatiles in critical, comparative analysis of floral scent chemistry based on a molecular phylogenetic tree can be useful to find (a) key volatiles affecting flower-pollinator interactions.

The authors thank Richard B. Figlar for useful advice, and the Instituto de Ecología, Xalapa, Mexico for providing funds and facilities to collect floral scents of Magnoliaceae in Mexico. This study was financially supported by JSPS Research Fellowships for Young Scientists.

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APPENDIX. List of taxa and accession numbers of DNA sequence used in this study (Azuma *et al.* 2001a, Kim *et al.* 2001). Taxonomic treatment of *Magnolia* follows Figlar & Nooteboom (2004).

Taxa	*trnK* (incl. *matK*)	*psbA-trnH*	*atpB-rbcL*	*ndhF*
Magnoliaceae				
Subf. Magnolioideae				
Genus *Magnolia*				
Subg. *Magnolia*				
Sect. *Magnolia*				
M. virginiana	AB020988	AB021018	AB021048	AF107939
M. grandiflora	AB020990	AB021020	AB021050	AF107940
M. guatemalensis	AB020991	AB021021	AB021051	AF107941
M. iltisiana	AB055520	AB055551	AB055569	AF216258
M. schiedeana	AB055550	AB055568	AB055586	AF221498
M. sharpii	AB020993	AB021023	AB021053	AF107942
M. tamaulipana	AB020994	AB021024	AB021054	AF107943
Sect. *Gwillimia*				
M. coco	AB021004	AB021034	AB021064	AF107916
M. delavayi	AB021005	AB021035	AB021065	AF107917
M. liliifera	AB021013	AB021043	AB021073	AF107919
Sect. *Talauma*				
M. dodecapetala	AB055535	AB055561	AB055579	AF119338
M. mexicana	AB055536	AB055562	AB055580	AF216263
M. portoricensis	AB055523	AB055554	AB055572	AF216254
M. splendens	AB055524	AB055555	AB055573	AF216259
Sect. *Manglietia*				
M. conifera	AB055541	AB055564	AB055582	AF107984
M. insignis	AB055543	AB055566	AB055584	AF107991
Sect. *Rytidospermum*				
M. obovata	AB020999	AB021029	AB021059	AF107925
M. officinalis	AB021000	AB021030	AB021060	AF107926
M. tripetala	AB021001	AB021031	AB021061	AF107928
M. sieboldii	AB021002	AB021032	AB021062	AF107933
M. wilsonii	AB055527	AB055558	AB055576	AF107934

APPENDIX. continued

Taxa	*trnK* (incl. *matK*)	*psbA-trnH*	*atpB-rbcL*	*ndhF*
Sect. *Auriculata*				
M. fraseri	AB020995	AB021025	AB021055	AF216256
Sect. *Macrophylla*				
M. macrophylla	AB020997	AB021027	AB021057	AF107923
M. dealbata	AB055525	AB055556	AB055574	AF107921
Subg. *Yulania*				
Sect. *Yulania*				
M. kobus	AB021008	AB021038	AB021068	AF107954
M. salicifolia	AB021009	AB021039	AB021069	AF107955
M. stellata	AB021010	AB021040	AB021070	AF107956
M. denudata	AB021007	AB021037	AB021067	AF107949
M. liliiflora	AB021012	AB021042	AB021072	AF107960
M. sargentiana	AB055532	AB055559	AB055577	AF107950
M. acuminata	AB021011	AB021041	AB021071	AF107958
Sect. *Michelia*				
M. figo	AB021015	AB021045	AB021075	AF107977
M. macclurei	AB055546	AB055567	AB055585	AF107967
Subg. *Gynopodium*				
Sect. *Gynopodium*				
M. nitida	AB021006	AB021036	AB021066	AF107935
Subf. Liriodendroideae				
Genus *Liriodendron*				
L. chinense	AB021016	AB021046	AB021076	AF107996
L. tulipifera	AB021017	AB021047	AB021077	AF107997

Received July 8, 2004 ; accepted August 8, 2004